

(7Z,9E)-2-METHYL-7,9-OCTADECADIENE: A SEX PHEROMONE COMPONENT OF *Lymantria bantaizana*^S

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Abstract—Our objective was to identify the sex pheromone of *Lymantria bantaizana* (Lepidoptera: Lymantriidae) whose larvae feed exclusively on walnut, *Juglans* spp., in China, and Japan. Coupled gas chromatographic–electroantennographic detection (GC-EAD) analyses of pheromone gland extracts revealed a single EAD-active component. Retention index calculations of this compound on four GC columns suggested that it was a methyl-branched octadecadiene with conjugated double bonds. In GC-EAD analyses of 2-methyloctadecenes, (Z)-2-methyl-7-octadecene and (E)-2-methyl-7-octadecene elicited the strongest antennal responses, suggesting that the double bond positions were at C7 and C9. In comparative GC-EAD analyses of pheromone gland extract and stereoselectively synthesized isomers (E,E; E,Z; Z,E; Z,Z) of 2-methyl-7,9-octadecadiene, the (E,Z)- and (Z,E)-isomer had retention times identical to that of the candidate pheromone, but only the latter isomer elicited strong EAD activity. Results of field experiments in Japan substantiated that (7Z,9E)-2-methyl-7,9-octadecadiene is the *L. bantaizana* sex pheromone, a compound previously unknown in the

^S While this manuscript was in press, a recent taxonomic revision of the world's *Lymantria* spp. proposed that *Lymantria bantaizana* should be more properly referred to as *Lymantria* (*Spinotria*) *griseascens bantaizana* Matsumura, 1933 (Schintlmeister, A. 2004. Taxonomy of the Genus *Lymantria* Hübner (1819) (Lepidoptera: Lymantriidae). *Quadrifina* 7:1–248).

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Lepidoptera. Detection surveys in North America for exotic Eurasian forest defoliators could include traps baited with the *L. bantaizana* pheromone.

Key Words—*Lymantria bantaizana*, *Lymantria dispar*, *Lymantria monacha*, *Lymantria fumida*, *Lymantria mathura*, *Lymantria xylina*, (7Z,9E)-2-methyl-7,9-octadecadiene, sex pheromone.

INTRODUCTION

Lymantria bantaizana Matsumura (Lepidoptera: Lymantriidae) is a little-known species found in Japan, Korea, and northern China (Inoue, 1957; Nam and Kim, 1981; Zhao, 1982). Larvae feed on walnut, *Juglans* spp., in China (Zhao, 1982) and Japan (Gotoh et al., 2004). Unlike all congeners that overwinter in the egg stage, *L. bantaizana* overwinter as caterpillars (Yasunori Kishida, Setagaya-ku, Tokyo, personal communication, August 28, 1997; Gotoh et al., 2004). The flight season of adults is from late June to early August in Japan (Inoue, 1957; Gotoh et al., 2004). Males had not been captured in traps baited with any synthetic pheromone known to date for *Lymantria* spp. When a gravid female was light-trapped in Japan and neonate larvae that hatched from her eggs could be reared to adults, the opportunity arose to identify the pheromone of this unusual *Lymantria* species. We report here the main component of this sex pheromone.

METHODS AND MATERIALS

Laboratory Analyses of Pheromone Extract and General Instrumentation.

Abdominal tips with the pheromone gland of calling (pheromone-emitting) 1-day-old virgin female *L. bantaizana* were removed and extracted for 5–20 min in high-performance liquid chromatography (HPLC)-grade hexane. Aliquots of pheromone gland extract and synthetic standards were analyzed by coupled gas chromatographic–electroantennographic detection (GC-EAD; Arn et al., 1975) and coupled GC-mass spectrometry (MS), employing equipment and procedures previously described in detail (Gries et al., 2002a). Relative EAD activities of 2-methyloctadecenes (produced by Wittig reactions from corresponding aldehydes and ylids) with (*E*)- or (*Z*)-double-bond geometry were determined in replicated ($N = 3$) GC-EAD analyses, using for each antennal preparation four isomers [(*E*3,*E*4,*E*5,*Z*7); (*Z*3,*Z*4,*Z*5,*Z*7); (*E*6,*E*7,*E*8,*Z*7); (*Z*6,*Z*8,*Z*9,*Z*7); or (*E*9,*E*10,*Z*10,*Z*7)] injected individually in split mode at 30-sec intervals and chromatographed isothermally (200°C; DB-5 column J&W Scientific). Nuclear magnetic resonance (NMR) spectroscopy of synthetic compounds was conducted on Bruker 300 (at 300 MHz for ^1H and 75 MHz for ^{13}C) and Varian AS500

(at 499.77 MHz for ^1H and 125.68 MHz for ^{13}C) spectrometers, with chemical shifts reported in ppm relative to TMS (^1H , δ 0.00) and CDCl_3 (^{13}C , δ 77.00). High-performance liquid chromatography (HPLC) of synthetic compounds employed a Waters LC 626 chromatograph equipped with a Waters 486 variable wavelength UV/visible detector set to 210 nm, HP Chemstation software (Rev.A.07.01), and a reverse phase Nova-Pak C18 column (60 Å, 4 μm ; 3.9 \times 300 mm, Waters) eluted with acetonitrile (1 ml/min).

Syntheses

(7Z,9E)-2-Methyl-7,9-octadecadiene (**4**) (Figure 1, Scheme 1). A solution (4.80 ml) of butyllithium in hexane (2.5 M, 12 mmol) was added dropwise to a stirred solution of 5-chloro-1-pentyne (**1**) (Aldrich Chem. Co.; 1.06 ml, 10 mmol) in 50 ml of THF under argon at -78°C . After 30 min, the mixture was warmed to -65°C , and 2.70 g (12 mmol) of anhydrous ZnBr_2 was added. After 45 min at -65°C , the mixture was warmed to -45°C , and a freshly prepared

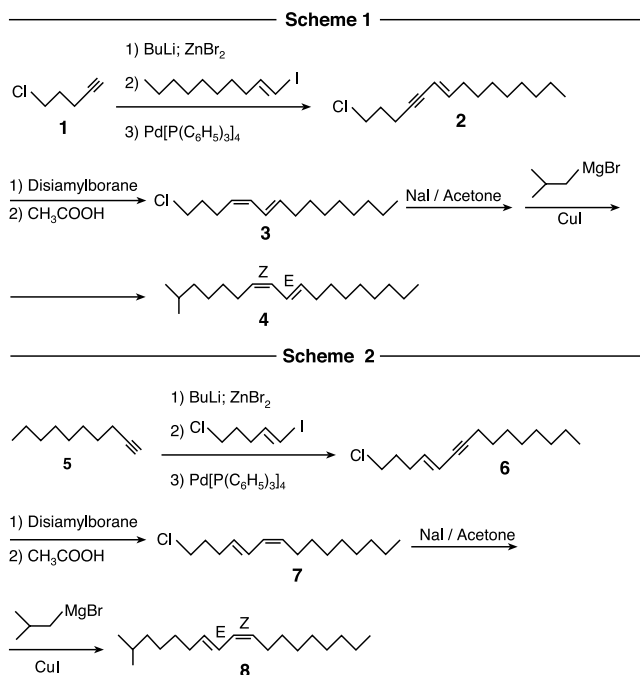


FIG. 1. Syntheses of the (Z,E)-isomer (Scheme 1) and (E,Z)-isomer (Scheme 2) of 2-methyl-7,9-octadecadiene.

solution of 6.00 mmol of 1-iodo-(*E*)-1-decene [from 1-decyne, DIBAL and iodine (Gardette et al., 1984)] in 20 ml THF was added, followed immediately by addition in one portion of tetrakis(triphenylphosphine)palladium (0.60 g, 0.50 mmol) in 20 ml of THF. After 30 min at -40 to -45°C , the reaction mixture was warmed to and kept at 20°C for 1 hr. The reaction was then quenched with 100 ml of saturated aq. NH_4Cl , extracted with ether (3×50 ml), dried overnight with anhydrous Na_2SO_4 , and concentrated *in vacuo*. Flash column chromatography (50 g SiO_2 , hexane as eluent) afforded 1.42 g (5.28 mmol, 88% yield) of (*E*6)-1-chloropentadeca-6-en-4-yne (**2**). ^1H NMR (CDCl_3) δ : 0.88 (t, 3H, $J = 6.8$ Hz); 1.22–1.40 (m, 12H); 1.97 (quint. 2H, H-2, $J = 6.7$ Hz); 2.07 (q, 2H, H-8, $J = 7.3$ Hz); 2.48 (dt, 2H, H-3, $J = 2.0, 6.7$ Hz); 3.65 (t, 2H, H-1, $J = 6.7$ Hz); 5.43 (td, 1H, H-6, $J = 2.0, 15.6$ Hz); 6.06 (td, 1H, H-7, $J = 7.3, 15.6$ Hz). ^{13}C NMR (CDCl_3) δ : 14.08, 16.78, 22.65, 28.77, 29.09, 29.22, 29.39, 31.51, 31.85, 32.96, 43.73, 80.18, 86.24, 109.39, 144.09. Anal. calcd. for $\text{C}_{15}\text{H}_{25}\text{Cl}$ (%): C 74.81, H 10.46, found C 74.51, H 10.42.

A solution of **2** (1.32 g, 4.91 mmol) in 50 ml THF was added slowly to freshly prepared (Zweifel and Poiston, 1970) and cooled (-10°C) disiamylborane (6.50 mmol). After stirring for 2.5 hr at 0°C , the mixture was warmed to and kept at room temperature for 5 hr. It was then treated with 6 ml of glacial acetic acid and stirring for 36 hr. Then, 100 ml of water were added, and the reaction mixture was extracted with hexane. Extracts were washed with saturated aq. NaHCO_3 and brine, dried with anhydrous MgSO_4 , and concentrated *in vacuo*. Flash chromatography afforded (*Z*4,*E*6)-1-chloropentadeca-4,6-diene (**3**) (1.22 g, 4.28 mmol, 87% yield, 95% pure by GC). A small sample of **3** was further purified by flash chromatography for spectroscopic analysis. ^1H NMR (CDCl_3) δ : 0.88 (t, 3H, $J = 6.9$ Hz); 1.25–1.45 (m, 12H); 1.86 (tt, 2H, H-2, $J = 6.6, 6.9$ Hz); 2.10 (tdd, 2H, H-8, $J = 6.9, 7.5, 1.3$ Hz); 2.33 (tdd, 2H, H-3, $J = 6.9, 7.5, 1.3$ Hz); 3.55 (t, 2H, H-1, $J = 6.6$ Hz); 5.24 (dt, 1H, H-4, $J = 10.8, 7.5$); 5.69 (dt, 1H, H-7, $J = 15.0, 7.5$ Hz); 6.01 (dd, 1H, H-5, $J = 10.8, 11.1$); 6.30 (dddt, 1H, H-6, $J = 11.1, 15.0, 1.3, 1.3$ Hz). ^{13}C NMR (CDCl_3) δ : 14.09, 22.65, 24.80, 29.22, 29.25, 29.30, 29.44, 31.86, 32.43, 32.85, 44.42, 125.17, 127.24, 130.17, 135.71. Anal. calcd. for $\text{C}_{15}\text{H}_{27}\text{Cl}$ (%): C 74.19, H 11.21, found C 73.85, H 11.02. Without further purification, **3** was dissolved in 50 ml of acetone, and 4.50 g of NaI (30 mmol) were added. After refluxing the mixture for 24 hr, 100 ml of water were added, and the resulting iodide was extracted with hexanes (3×50 ml). The combined extracts were washed with 2% aq. $\text{Na}_2\text{S}_2\text{O}_3$ and brine, dried, concentrated, and, at -23°C , added to 50 ml of a THF solution of isobutylmagnesium bromide (13.2 mmol) and CuI (0.25 g, 1.32 mmol). After 20 min, the reaction mixture was allowed to warm to room temperature, quenched with aq. NH_4Cl , extracted with hexane, washed with brine, dried, concentrated *in vacuo*, and filtered through 10 g of silica in hexane. Concentration yielded (7*Z*,9*E*)-2-methyl-7,9-octadecadiene (**4**) (0.81 g, 3.00 mmol, 70% yield based

on **2**, 97% pure based on GC). The (Z,Z)-isomer contaminant was removed by HPLC, affording >99% geometrically pure **4**. ^1H NMR (C_6D_6) δ : 0.93 (d, 6H, $J = 6.4$ Hz); 0.94 (t, 3H, $J = 6.8$ Hz); 1.21–1.45 (m, 18H); 1.51 (m, 1H, H-2); 2.14 (td, 2H, H-11, $J = 7.1, 6.9$ Hz); 2.25 (tdd, 2H, H-6, $J = 7.5, 7.5, 1.3$ Hz); 5.44 (dt, 1H, H-7, $J = 10.8, 7.5$ Hz); 5.73 (dt, 1H, H-10, $J = 15.0, 7.1$ Hz); 6.21 (dd, 1H, H-8, $J = 10.8, 10.8$ Hz); 6.57 (dddt, 1H, H-9, $J = 15.0, 10.8, 1.3, 1.3$ Hz). ^{13}C NMR (CDCl_3) δ : 14.11, 22.65 (2), 22.68, 27.03, 27.72, 27.95, 29.26, 29.29, 29.42, 29.49, 29.99, 31.89, 32.90, 38.86, 125.59, 128.58, 130.07, 134.68; MS: 41 (42), 55 (25), 67 (100), 81 (95), 95 (66), 109 (36), 123 (23), 137 (8), 208 (5), 264 (13). Anal. calcd. for $\text{C}_{19}\text{H}_{36}$ (%): C 86.28, H 13.72, found C 86.34, H 13.77.

(7E,9Z)-2-Methyl-7,9-octadecadiene (**8**). Compound **8** (Figure 1, Scheme 2) was synthesized analogous to **4**, starting from 1-decyne (**5**), which was coupled in the presence of tetrakis(triphenylphosphine)palladium with (E)-5-chloro-1-iodo-1-pentene to produce (E)-1-chloropentadeca-4-en-6-yne (**6**). Compound **6** was treated with disiamylborane (as above) to yield (4E,6Z)-1-chloropentadeca-4,6-diene (**7**) which was converted in two steps to (7Z,9E)-2-methyl-7,9-octadecadiene (**8**) which was purified by HPLC. ^1H NMR (C_6D_6) δ : 0.93 (d and t 9H, $J = 6.4, 6.8$ Hz); 1.20–1.45 (m, 18H); 1.51 (m, 1H, H-2); 2.13 (td, 2H, H-6, $J = 7.1, 6.8$ Hz); 2.26 (tdd, 2H, H-11, $J = 7.5, 7.5, 1.3$ Hz); 5.45 (dt, 1H, H-10, $J = 10.8, 7.5$ Hz); 5.73 (dt, 1H, H-7, $J = 15.0, 7.1$ Hz); 6.21 (dd, 1H, H-9, $J = 10.8, 10.8$ Hz); 6.57 (dddt, 1H, H-8, $J = 15.0, 10.8, 1.3, 1.3$ Hz). MS: 41 (40), 55 (26), 67 (100), 81 (91), 95 (62), 109 (34), 123 (21), 137 (11), 208 (5), 264 (12).

(7E,9E)-2-Methyl-7,9-Octadecadiene (**12**). 2,5-Dihydrothiophene-1,1-dioxide (**9**) (Aldrich; 25.0 g, 210 mmol) in 300 ml of dry THF was placed under argon in a three-neck 1-l flask equipped with two dropping funnels. The mixture was cooled to -78°C and stirred vigorously while lithium bis(trimethylsilyl) amide (70 ml of 1 M solution in THF) and 30 ml of THF were added in two portions via one dropping funnel. Via the second dropping funnel, a mixture of 5-methyl-1-iodohexane [5-methyl-1-chlorohexane (available from previous work) was refluxed with sodium iodide in acetone] (16.0 g, 70 mmol), 45 ml of hexamethylphosphoramide (HMPA), and 140 ml of THF was added in 15 min (Yamada et al., 1983). The reaction mixture was then warmed to room temperature, and the reaction was quenched with 100 ml of water and extracted (3×100 ml) with ethyl acetate/hexane (1:1). Extracts were washed with water, dried (MgSO_4), and concentrated *in vacuo*. Flash column chromatography of the residue with ether/hexane as eluent [gradually increasing (5–30%) the ether content] afforded the pure mono-substituted sulfone (**10**) (6.10 g, 28.2 mmol, 40% yield based on the iodide). The process was repeated with **10** and excess 1-iodooctane (6.32 ml, 35 mmol), 30 mmol of lithium bis(trimethylsilyl)amide, and 26 ml of HMPA, affording after work-up and separation 2,5-disubstituted sulfone (**11**) (3.30 g, 10 mmol, 35.5% yield based on **10**). Sulfone **11** (1 g, 3.0

mmol) was refluxed in 20 ml of 1-propanol with 0.42 g of K_2CO_3 for 2 hr (Yamada et al., 1983) until desulfonylation was complete and (7*E*,9*E*)-2-methyl-7,9-octadecadiene (**12**) had formed. Compound **12** (0.80 g, 89% yield) (Figure 2, Scheme 3) was extracted with hexane from the reaction mixture and purified by flash chromatography. (*E,Z*)- and (*Z,E*)-isomer contaminants (5%) were removed by HPLC, affording >99% geometrically pure **12**. 1H NMR (C_6D_6) δ : 0.92 (d, 6H, $J = 6.4$ Hz); 0.94 (t, 3H, $J = 6.8$ Hz); 1.20–1.48 (m, 18H); 1.51 (m, 1H, H-2); 2.11 (two td, 4H, H-6 and H-11, $J = 7.0, 7.0$ Hz); 5.66 (m, 2H, H-7 and H-10); 6.20 (m, 2H, H-8 and H-9). MS: 41 (38), 55 (21), 67 (100), 81 (99), 95 (67), 109 (37), 123 (23), 137 (13), 208 (5), 264 (22).

(7*Z*,9*Z*)-2-Methyl-7,9-octadecadiene (**18**). To a solution of **5** (3.68 ml, 20 mmol) in 50 ml of THF under argon at $-70^\circ C$, 9.2 ml (23 mmol) of 2.5 M BuLi in hexane were added, and 3.0 g of paraformaldehyde 30 min later. The mixture was allowed to warm to room temperature; 5 hr later, the reaction was quenched with water. 2-Undecyn-1-ol (**13**) was extracted (2×75 ml) with ether hexane (1:1). Extracts were washed with water and brine, dried, and concentrated. Alcohol **13** (93% pure based on GC) was then hydrogenated with P2-nickel catalyst (Brown and Ahuja, 1973) to yield (*Z*)-2-undecen-1-ol (**14**) (3.0 g, 85% yield, 96% pure based on GC). Alcohol **14** (2.15 g, 12.6 mmol) was epoxidized in 25 ml of dichloromethane with *m*-chloroperbenzoic acid (30 mmol, 8.63 g, 60% pure) at $0^\circ C$ for 5 hr. The reaction mixture was warmed to room temperature, water (10 ml) was added, and the product was extracted with ether (50 ml). The extract was washed (2N NaOH, water, brine), dried ($MgSO_4$), and concentrated. Flash chromatography of the crude product afforded *cis*-(2,3)-epoxy-1-undecanol (**15**) (2.30 g, 98% pure based on GC). Alcohol **15** (1 g, 5.37 mmol) was added to a stirred mixture (10 min at $-60^\circ C$) of oxalyl chloride (0.95 ml, 10.8 mmol) and DMSO (1.53 ml, 21.6 mmol; Mancuso et al., 1978). After 20 min, 12 ml of triethylamine were added. After 30 min, the reaction mixture was allowed to warm to room temperature, quenched with water, and extracted with ether. The organic layer was washed (water, brine), dried ($MgSO_4$), and concentrated *in vacuo* to afford the epoxy-aldehyde (**16**). Without further purification, **16** was placed in 50 ml of dry THF and immediately added dropwise to the ylid freshly prepared from 5.02 g (10 mmol) of 6-methylheptyltriphenylphosphonium iodide [synthesized from 6-methyl-1-chloroheptane (available from previous work) by refluxing it with NaI in acetone, and subsequent reflux of the formed iodide with triphenylphosphine in toluene] in 40 ml of THF and 11 ml (11 mmol, 1 M solution in THF) of sodium bis(trimethylsilyl)amide] at $-70^\circ C$. The reaction mixture was allowed to gradually warm to room temperature, quenched with 100 ml of water-methanol (1:1), extracted with hexane, and purified by flash chromatography (5% ether in hexane) to afford (*Z*)-2-methyl-*cis*-9,10-epoxy-7-octadecene (**17**) [0.89 g, 3.17 mmol, 59% yield based on **15**, with 10% of the (*E*)-isomer]. (7*Z*,9*Z*)-2-methyl-

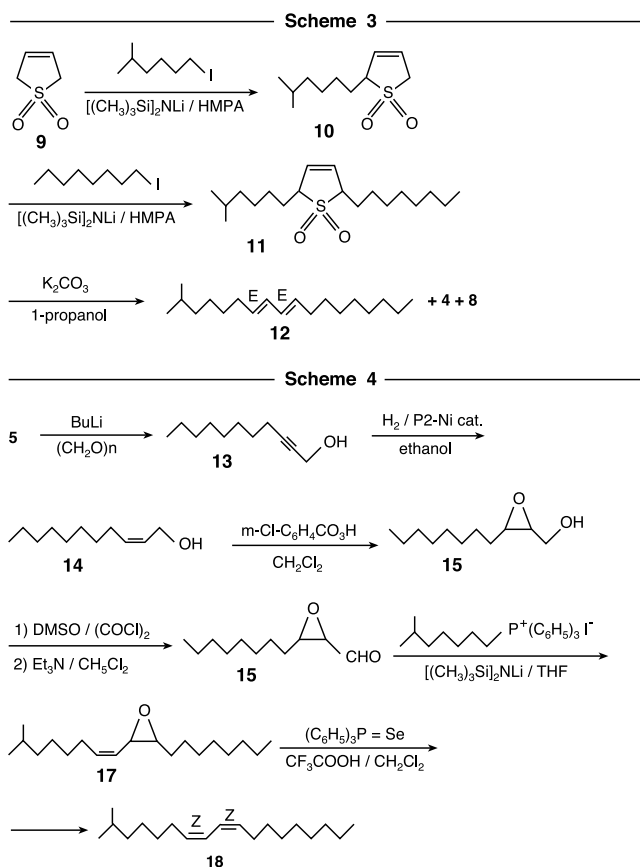


FIG. 2. Syntheses of the (*E,E*)-isomer (Scheme 3) and (*Z,Z*)-isomer (Scheme 4) of 2-methyl-7,9-octadecadiene.

7,9-octadecadiene (**18**) (Figure 2, Scheme 4) was obtained by treating 0.29 g (1.04 mmol) of **17** with 2.00 mmol of freshly prepared triphenylphosphineselenium in dichloromethane (Clive, 1978) in the presence of trifluoroacetic acid (1 ml) for 20 min. Solvents were removed *in vacuo* from the reaction mixture, and 50 ml of pentane were added. The mixture was filtered through silica (10 g) which was washed with an additional 20 ml of pentane. After concentration, the resulting mixture of **18** (80%, 0.23 g, 83% yield) and some **8** (Scheme 2) was purified by HPLC, affording >99% pure **18**. ^1H NMR (C_6D_6) δ : 0.92 (d, 6H, $J = 6.4$ Hz); 0.92 (t, 3H, $J = 6.8$ Hz); 1.21–1.45 (m, 18H); 1.50 (m, 1H, H-2); 2.23 (two td, 4H, H-6 and H-11, $J = 7.1, 7.1$ Hz); 5.56 (m, 2H, H-7 and H-10); 6.50

(m, 2H, H-8 and H-9). MS: 41 (45), 55 (26), 67 (100), 81 (90), 95 (64), 109 (33), 123 (21), 137 (10), 208 (5), 264 (17).

Field Experiments. Candidate pheromone components were field tested in the Iwate University forest (Takizawa Iwate Prefecture; N39°46.5', E141°9.14') stocked with mainly oak trees, *Quercus* spp., and some walnut trees, *Juglans* spp., and in the Mizunashi farming valley (N39°53.3', E141°18.18') with riparian Siebold walnut trees. At 15- to 20-m intervals, sticky 2-l milk carton traps (Gray et al., 1984) were suspended from trees 1.5 m above ground in complete randomized blocks. Traps were baited with a gray sleeve stopper (The West Co., Lionville, PA, USA) impregnated with test chemicals in HPLC-grade hexane. Experiment 1 tested all four geometrical isomers singly and in quaternary combination, whereas experiment 2 tested the (*E,Z*)- and (*Z,E*)-isomers of 2-methyl-7,9-octadecadiene singly and combined. Experiment 3 tested the diel periodicity of response by male *L. bantaizana*, by baiting traps ($N = 20$) with synthetic (7*Z*,9*E*)-2-methyl-7,9-octadecadiene (50 µg) and by recording captures of males in 1-hr intervals during the night of July 29, 2001 near Tamayama Village (N39°52.16', E141°26.16').

Trap catch data in experiments 1 and 2 were analyzed by nonparametric analyses of variance (Friedman's test) followed by comparison of means by Scheffé test (Zar, 1984; SAS/STAT, 1988). In all analyses, $\alpha = 0.05$.

RESULTS AND DISCUSSION

GC-EAD analyses of pheromone gland extract (Figure 3) on four GC columns revealed a single candidate pheromone component (CPC) that consistently elicited responses from male antennae. CPC occurred at quantities too low to obtain a mass spectrum. Thus, it was identified based on retention time matches and comparative antennal responses of the insect-produced compound and synthetic standards.

Retention indices (RI, relative to alkane standards; van den Dool and Kratz, 1963) of CPC were similar on four nonpolar and polar GC columns (DB-5: RI = 1884; DB-210: RI = 1942; DB-23: RI = 2015; SP-1000: RI = 2002) indicative of a hydrocarbon. Synthetic (*Z*)-2-methyl-7-octadecene (2me-Z7-18Hy), a pheromone component of *L. monacha* (Grant et al., 1996; Gries et al., 1996), *L. fumida* (Schaefer et al., 1999), and *L. lucescens* and *L. serva* (Gries et al., 2002b) elicited strong responses from antennae of male *L. bantaizana*, suggesting that it resembled the CPC. The fact that 2me-Z7-18Hy elicited the strongest EAD response of all tested 2-methyloctadecenes (Figure 4) substantiated this conclusion. Higher retention indices of CPC than of 2me-Z7-18Hy on several GC columns (DB-5: + 42; DB-210: + 82; DB-23: + 138) further

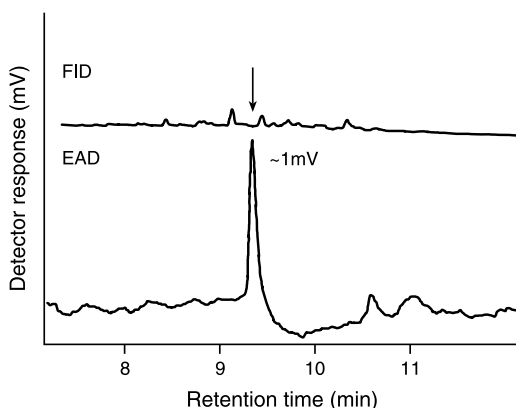


FIG. 3. Flame ionization detector (FID) and electroantennographic detector (EAD: male *L. bantaizana* antenna) responses to one female equivalent (FE) of pheromone extract from female *L. bantaizana*. Chromatography: DB-5 column; splitless injection; injector and FID detector: 240°C; temperature program: 100°C (1 min), then 20°C/min to 190°C (8 min).

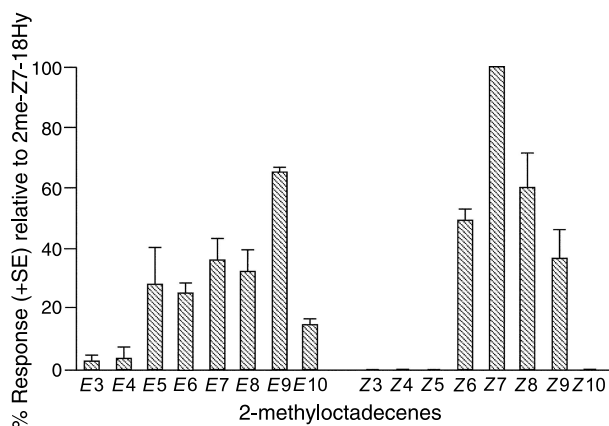


FIG. 4. Relative EAD activity of 2-methyloctadecenes with (*E*)- or (*Z*)-double bond geometry, as determined in replicated ($N = 3$) GC-EAD analyses. For each antennal preparation, four isomers [(*E*3,*E*4,*E*5,*Z*7; (*Z*3,*Z*4,*Z*5,*Z*7); (*E*6,*E*7,*E*8,*Z*7); (*Z*6,*Z*8,*Z*9,*Z*7); or (*E*9,*E*10,*Z*10,*Z*7)] were tested, injected individually in split mode at 30-sec intervals and chromatographed isothermally (200°C; DB-5 column).

suggested that CPC had conjugated double bonds. Taking into account that (*E*)-2-methyl-9-octadecene elicited the second strongest EAD response (Figure 4), we hypothesized that the second double bond was at C9. Strong EAD activity of one geometrical isomer in the 4-isomer (*E,E*; *E,Z*; *Z,E*; *Z,Z*) mixture of 2-methyl-7,9-octadecadiene confirmed this hypothesis. In comparative GC-EAD analyses of pheromone gland extract and synthetic 2-methyl-7,9-octadecadienes, the (*E,Z*)- and (*Z,E*)-isomers had retention times identical with those of the candidate pheromone, but only the latter isomer elicited strong EAD responses.

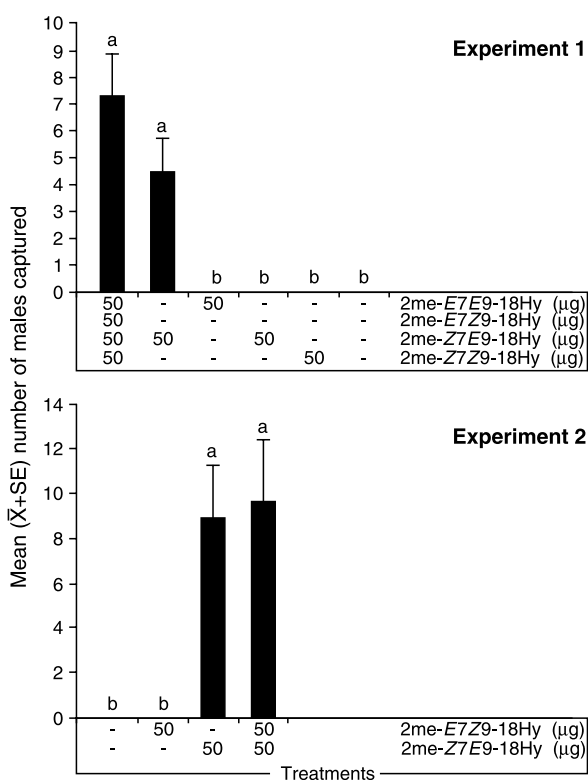


FIG. 5. Mean (\pm SE) number of male *L. bantaizana* captured in sticky 2-l milk carton traps baited with geometrical isomers (*E,E*; *E,Z*; *Z,E*; or *Z,Z*) of 2-methyl-7,9-octadecadiene. Experiment 1: July 2–August 11, 2003, $N = 6$; Experiment 2: July 5–August 19, 2002, $N = 10$. Bars in each experiment with different letter superscripts are significantly different; nonparametric analysis of variance by ranks (Friedman's test) followed by comparison of means (Scheffé test), $P < 0.05$ (SAS/STAT, 1988).

In field experiments 1 and 2 (Figure 5), only the (Z,E)-isomer attracted males, whereas other isomers when added to the (Z,E)-isomer did not inhibit the males' responses. In experiment 3, ten males were captured between 24:00 and 01:00 hr and three males between 01:00 and 02:00 hr. This information about the diel periodicity of sexual communication in *L. bantaizana* will contribute to our ongoing studies as to how communication signals and time of signaling impart specificity to communication channels of five congeners (*Lymantria dispar*, *L. fumida*, *L. lucescens*, *L. mathura*, *L. monacha*) in the complex *Lymantria* communities of Japan.

Following the pioneering discovery of (7R,8S)-*cis*-7,8-epoxy-2-methyloctadecane [(+)-disparlure] as the pheromone of gypsy moth, *L. dispar* (Bierl et al., 1970; Klimetzek et al., 1976; Cardé et al., 1977; Plimmer et al., 1977), there is mounting evidence that sex pheromones of *Lymantria* spp. are more diverse than previously realized. (7R,8S)-*cis*-7,8-Epoxyoctadecane serves as a synergistic pheromone component of nun moth, *L. monacha*, in central Europe (Gries et al., 1996), and as the major pheromone component of *L. monacha* in Japan (Gries et al., 2001). (3Z,6Z,9R,10S)-*cis*-9,10-Epoxyonadecadiene and (3Z,6Z,9S,10R)-*cis*-9,10-epoxyonadecadiene at a 1:4 ratio are sex pheromone components of pink gypsy moth, *L. mathura*, in Japan (Gries et al., 1999a), and (7R,8S)-*cis*-7,8-epoxyeicosane is the pheromone of Casuarina moth, *Lymantria xylina*, in Taiwan (Gries et al., 1999b). (Z)-2-methyl-7-octadecene is a synergistic pheromone component of both *L. monacha* in Central Europe (Grant et al., 1996; Gries et al., 1996) and Japan (Gries et al., 2001) and of *L. fumida* in Japan (Schaefer et al., 1999). The same compound constitutes the pheromone of *L. lucescens* in Japan and *L. serva* in Taiwan (Gries et al., 2002b). Finally, here we show that a methylated diene hydrocarbon is part of the diverse sex pheromones in *Lymantria* spp.

Considering that *L. bantaizana* larvae defoliate walnut trees (an important nut crop in North America) and that *L. bantaizana* is as likely as Asian *L. mathura*, *L. dispar*, and *L. monacha* to be introduced into North America, traps baited with synthetic *L. bantaizana* pheromone could be included in detection surveys for exotic *Lymantria* spp. Commercial trap lures containing a 1:1 mixture of (E,Z)- and (Z,E)-isomers will be cheap because such a mixture is readily obtainable from sulfone **11** by desulfonylation at 110°C in a nonpolar solvent rather than the polar solvent shown in Scheme 3 (Yamada et al., 1983).

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REFERENCES

- ARN, H., STÄDLER, E., and RAUSCHER, S. 1975. The electroantennographic detector—a selective and sensitive tool in the gas chromatographic analysis of insect pheromones. *Z. Naturforsch.* 30c:722–725.
- BIERL, B. A., BEROZA, M., and COLLIER, C. W. 1970. Potent sex attractant of the gypsy moth: Its isolation, identification and synthesis. *Science* 170:87–89.
- BROWN, A. L. and AHUJA, V. K. 1973. “P2-nickel” catalyst with ethylenediamine, a novel system for highly stereospecific reduction of alkynes to *cis*-olefins. *J. Chem. Soc., Chem. Commun.* 553–554.
- CARDÉ, R. T., DOANE, C. C., BAKER, T. C., IWAKA, S., and MARUMO, S. 1977. Attractancy of optically active pheromone for male gypsy moth. *Environ. Entomol.* 6:768–772.
- CLIVE, D. L. J. 1978. Modern organoselenium chemistry. *Tetrahedron* 34:1049–1132.
- GARDETTE, M., JABRI, N., ALEXAKIS, A., and NORMANT, J. F. 1984. General methodology for the synthesis of conjugated dienic insect pheromones. *Tetrahedron* 40:2741–2750.
- GRANT, G. G., LANGEVIN, D., LIŠKA, J., KAPITOLA, P., and CHONG, J. M. 1996. Olefin inhibitor of gypsy moth, *Lymantria dispar*, is a synergistic pheromone component of nun moth, *L. monacha*. *Naturwissenschaften* 83:328–330.
- GRAY, T. G., SLESSOR, K. N., SHEPHERD, R. F., GRANT, G. G., and MANVILLE, J. F. 1984. European pine shoot moth, *Rhyacionia buoliana* (Lepidoptera: Tortricidae): Identification of additional pheromone components resulting in an improved lure. *Can. Entomol.* 116:1525–1532.
- GOTOH, T., SCHAEFER, P. W., and DOI, N. 2004. Food plants and life cycle of *Lymantria bantaizana* Matsumura (Lepidoptera: Lymantriidae) in northern Honshu, Japan. *Entomol. Sci.* 7:123–129.
- GRIES, G., GRIES, R., KHASKIN, G., SLESSOR, K. N., GRANT, G. G., LIŠKA, J., and KAPITOLA, P. 1996. Specificity of nun and gypsy moth sexual communication through multiple-component pheromone blends. *Naturwissenschaften* 83:382–385.
- GRIES, G., GRIES, R., SCHAEFER, P. W., GOTOH, T., and HIGASHIURA, Y. 1999a. Sex pheromone components of pink gypsy moth, *Lymantria mathura*. *Naturwissenschaften* 86:235–238.
- GRIES, G., SCHAEFER, P. W., KHASKIN, G., HAHN, R., GRIES, R., and CHAO, J.-T. 1999b. Sex pheromone component of Casuarina moth, *Lymantria xylina*. *J. Chem. Ecol.* 25:2535–2545.
- GRIES, G., SCHAEFER, P. W., GRIES, R., LIŠKA, J., and GOTOH, T. 2001. Reproductive character displacement in *Lymantria monacha* from northern Japan? *J. Chem. Ecol.* 27:1163–1176.
- GRIES, R., KHASKIN, G., GRIES, G., BENNETT, R. G., KING, G. G. S., MOREWOOD, P., SLESSOR, K. N., and MOREWOOD, W. D. 2002a. (Z,Z)-4,7-Tridecadien-(S)-2-yl acetate: Sex pheromone of Douglas-fir cone gall midge, *Contarinia oregonensis*. *J. Chem. Ecol.* 28:2283–2297.
- GRIES, G., SCHAEFER, P. W., GRIES, R., FAN, Y. B., HIGASHIURA, Y., and TANAKA, B. 2002b. 2-Methyl-(Z)-7-octadecene: Sex pheromone of allopatric *Lymantria lucescens* and *L. serva*. *J. Chem. Ecol.* 28:469–478.
- INOUE, H. 1957. A revision of the Japanese Lymantriidae (II). *Jpn. J. Med. Sci. Biol.* 10:187–219.
- KLIMETZEK, D., LOSKANT, G., VITÉ, J. P., and MORI, K. 1976. Disparlure: Differences in pheromone perception between gypsy and nun moth. *Naturwissenschaften* 65:581–582.
- MANCUSO, A. J., HUANG, S.-L., and SWERN, D. 1978. Oxidation of long-chain and related alcohols to carbonyls by dimethyl sulfoxide “activated” by oxalyl chloride. *J. Org. Chem.* 43:2480–2482.
- NAM, S.-H. and KIM, C.-W. 1981. A synonymic list of tussock moths (Orgyidae: Lep.) in Korea. *Entomol. Res. Bull.* 8:73–100.
- PLIMMER, J. R., SCHWALBE, C. P., PASZEK, E. C., BIERL, B. A., WEBB, R. E., MARUMO, S., and IWAKIM, S. 1977. Contrasting effects of (+)- and (–)-enantiomers of disparlure for trapping native populations of the gypsy moth in Massachusetts. *Environ. Entomol.* 6:518–522.

- SAS/STAT. 1988. User's Guide, Release 6.03 Edition. SAS Institute, Cary, NC.
- SCHAEFER, P. W., GRIES, G., GRIES, R., and HOLDEN, D. 1999. Pheromone components and diel periodicity of pheromonal communication in *Lymantria fumida*. *J. Chem. Ecol.* 25:2305–2312.
- VAN DEN DOOL, H. and KRATZ, P. D. 1963. A generalization of the retention index system including linear temperature programmed gas–liquid partition chromatography. *J. Chromatogr.* 2:463–471.
- YAMADA, S., OHSAWA, H., SUZUKI, T., and TAKAYAMA, H. 1983. Stereoselective synthesis of (*E*)-, (*E,Z*)-, and (*E,E*)-conjugated dienes via alkylation of 3-sulfolenes as key step. *Chem. Lett. Chem. Soc. Jpn.* 1003–1006.
- ZAR, J. H. 1984. Biostatistical Analysis. Prentice-Hall, Englewood Cliffs, NJ.
- ZHAO, Z.-L. 1982. Lymantriidae, pp. 163–190, in *Iconographia Heterocerorum sinicorum*. II. Notodontidae, Lymantriidae, Arctiidae, Hypsiidae, Amatidae. Science Press, Beijing pls. 39–75. pp. 135–235.
- ZWEIFEL, G. and POISTON, N. L. 1970. Selective hydroboration of conjugated diynes with dialkylboranes. A convenient route to conjugated *cis*-enynes, α,β -acetylenic ketones, and *cis,cis*-dienes. *J. Am. Chem. Soc.* 92:4068–4071.